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Project Report: Transcriptomes of Permafrost Bacteria

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Project Progress

The Siberian permafrost is an extreme environment. Average temperatures of -10 to -12°C concentrate solutes to an $a_w = 0.89$ (5 osm) and limit diffusion of available nutrients to the cells. The overall goal of this project is to identify genes that are critical for bacteria to successfully inhabit this environment. One line of investigation is to identify and determine the functions of genes that are activated in response to low temperature and low water activity. In this project, we are monitoring gene expression at the transcriptome level. Taking advantage of our permafrost genome sequencing project, we have developed a 70 mer oligonucleotide array that probes expression of 1990 of the ~2100 genes encoded by *Psychrobacter* 273–4. Using the arrays, we have compared the transcriptomes of *Psychrobacter* 273–4 growing at 22 and 4°C . Changes in transcript levels were observed for about 22 genes involved in lipid biosynthesis, protein synthesis (ribosomal proteins), amino acid metabolism, and transport. The differential expression of transport genes supports physiological evidence that different carbon sources are utilized at the two temperatures. Most of the cold-regulated genes, however, were only up-regulated about 1.5 to 2 fold, indicating that *Psychrobacter* 273–4 may not be physiologically stressed growing at temperatures between 4 and 22°C . Experiments, therefore, are currently in progress to examine a wider range of growth temperatures including subzero temperatures.

The oligonucleotide arrays that we have made have also been used to examine changes in the transcriptome induced in response to low water activity. Low water activity presented a greater stress to *Psychrobacter* 273–4 than 4°C growth alone with 10% more differential gene expression. The majority of genes were down-regulated (2-fold or greater) when grown in the presence of 5% NaCl and are involved in cation transport, indicating a response to limit NaCl transport into the cell. Up-regulation of a gene predicted to be involved in cell shape correlates with the physiological determinations of decreased cell surface/volume in the presence of 5% NaCl. In addition, general stress response chaperones were up-regulated under both increased salinity and decreased temperatures. Several other genes with unknown functions were also up-regulated under both stresses, which may indicate their importance in

stress survival.

Highlights

- The transcriptome of *Psychrobacter* 273–4 displays differences at 22 and 4 °C, but the differences in transcript levels are small (1.5– to 2–fold) suggesting that these permafrost bacteria are not physiologically stressed at the relatively low temperature of 4 °C.
- Transcriptome responses of *Psychrobacter* 273–4 to increased osmotica show relatively small transcript changes, suggesting that 5% NaCl is not a stress condition in itself.
- Coupling low temperature and increased osmotica leads to transcriptome differences in chaperones, transporters, and antioxidants which may be important in permafrost in situ conditions.

Roadmap Objectives

- **Objective No. 5.1:** Environment–dependent, molecular evolution in microorganisms
- **Objective No. 5.3:** Biochemical adaptation to extreme environments
- **Objective No. 6.2:** Adaptation and evolution of life beyond Earth